Cornell iGEM Growth Experiments:
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9 strains x 3 metal concentrations x 3 replicates = 81 total

Required media components: LB, required antibiotic/s, respective metal, 1x arabinose.

Note: 1000x Arabinose solution is in a 50 mL tube in the black fridge.

Strains used:
pC13AS (BL21 expressing crtI, used as a BL21 control)
pA14R (CBP4 synthesized by genscript)
pC14I (MerT/merP in pSB1C3)
pC14K (NixA in pSB1C3)
AI+R (Metallothionein+CBP4)
AI+AG (Metallothionein + merT/merP)
AI+AF (Metallothionein + nixA)

Day 1:

1. Grow 15 mL overnight cultures of all strains in LB + respective antibiotics (Cm, Amp, CM+Amp) + 1x Arabinose

Note: these overnight cultures should not include metals. Also, you will only need one for each strain for a total number of 7 cultures.

Day 2:
1. Measure OD of each culture in UV spec.
2. Add 140 uL of appropriate media into wells of a 96 well plate **in the sterile hood.** Appropriate medias include LB, respective antibiotic/s, and the following metals at various concentrations:

pC14AS/Pb: 1mM Pb, .1mM Pb, .01mM Pb
pC14AS/Hg: 5uM Hg, .5 uM Hg, .05 uM Hg
pC14AS/Ni: 1mM Ni, .1 mM Ni, .01mM Ni
pA14R/Pb: 1mM Pb, .1mM Pb, .01mM Pb
pC14I/Hg: 5uM Hg, .5 uM Hg, .05 uM Hg
pC14K/Ni: 1mM Ni, .1 mM Ni, .01mM Ni
AI+R/Pb: 1mM Pb, .1mM Pb, .01mM Pb
AI+AG/HG: 5uM Hg, .5 uM Hg, .05 uM Hg
AI+AF/Ni: 1mM Ni, .1 mM Ni, .01mM Ni

Remember, all of the above need to be in triplicate! This will make a total of 81 wells of the plate filled. Be sure to organize them as well as you can!

Note: you should make more of each respective media in a 15mL or 2mL tube prior to the testing so that we will have the medias prepared if we want to repeat the experiment.

3. Calculate the cell inoculation volume (V) in uL for each experimental condition. You will calculate this by: V=1/(measured OD). For example if the OD is 1, the inoculation volume V will be 1uL into each well. This is to correct for initial cell density in each well.
4. Inoculate cells into the appropriate wells in the plate reader after the media has been added. **In the sterile hood**
5. Cover the plate evenly with parafilm or plate cover to prevent evaporation
6. Set plate reader temperature to 37C
7. Set experimental conditions for the plate reader:

- Go to basic protocols-->Basic kinetic protocol
- Wavelength: 600nm
- Shaking: 5s before each read
- Total time: 24 hours, read every hour
- Read full plate

8. Open the drawer and place the plate in the holder in the correct orientation. Plate should lightly "snap" into place. Close drawer.
9. Press read on the computer program
10. Wait until run is over and retrieve the data.